**Clinicians' interpretations of point of care urine culture vs laboratory culture results**: **analysis from the four-country POETIC trial of diagnosis of uncomplicated urinary tract infection in primary care**

**Short title:** Clinicians' interpretations of point of care urine culture vs laboratory culture results

**Article category:** Primary Care Epidemiology

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## Abstract

### Background
Urine culture at the point of care minimises delay between obtaining the sample and agar inoculation in a microbiology laboratory, and quantification and sensitivity results can be available more rapidly in primary care.

### Objective
To identify the degree to which clinicians' interpretations of a point-of-care-test (POCT) urine culture (Flexicult™ SSI-Urinary Kit) agrees with laboratory culture in women presenting to primary care with symptoms of uncomplicated urinary tract infections (UTI).

### Methods
Primary care clinicians used the Flexicult™-POCT, recorded their findings and took a photograph of the result, which was interpreted by microbiology laboratory technicians. Urine samples were additionally processed in routine care laboratories. Cross tabulations were used to identify important differences in organism identification, quantification and antibiotic susceptibility between these three sources of data. The influence of various laboratory definitions for UTI on culture were assessed.

### Results
Primary care clinicians identified 202/289 urine samples (69.9%) as positive for UTI using the Flexicult™-POCT, whereas laboratory culture identified 94-190 (32.5-65.7%) as positive, depending on definition thresholds. 82.9% of samples identified positive for *E. coli* on laboratory culture were also considered positive for *E. coli* using the Flexicult™ -POCT, and susceptibilities were reasonably concordant. There were major discrepancies between laboratory staff interpretation of Flexicult™ photographs, clinicians' interpretation of the Flexicult™ test, and laboratory culture results.

### Conclusion
Flexicult™-POCT overestimated the positivity rate of urine samples for UTI when laboratory culture was used as the reference standard. However, it is unclear whether point-of-care or laboratory based urine culture provides the most valid diagnostic information.

**249 words**

**Keywords** Urinary tract infection, Primary Health Care, Point of Care Test, Adult women, Antibiotic resistance, Diagnosis

**Background**

Nearly 40% of women suffer from symptoms of urinary tract infection (UTI) in their lifetime and UTIs account for about 15% of antibiotic prescriptions in primary care.[1,2] The majority of symptoms attributed to UTIs in otherwise healthy women presenting to primary care are accounted for by uncomplicated episodes of acute cystitis and urethritis. Nevertheless, they have a major impact on quality of life.[3] In line with current general practice guidelines, women with typical UTI symptoms are usually treated empirically without additional diagnostic testing.[4,5] This may result in both inappropriate and unnecessary antibiotic use.

Antibiotic resistance, especially in Gram-negative organisms that cause most UTIs, is increasing and is associated with antibiotic use. [6] The World Health Organization considers antimicrobial resistance to be one of the three greatest threats to human health. [7] Therefore, strategies to better target antibiotics to those who are most likely to benefit are needed.

There is currently no international gold standard for the microbiological diagnosis for UTI, including no consensus about the quantification of bacteria on urine culture that confirms a UTI.[8] General practitioners (GPs) frequently use a point of care test (POCT) such as urine dipstick or dip-slides. However, these approaches neither predict antibiotic response nor indicate antibiotic susceptibility, which are important to guide appropriate choice of antibiotic class.

The Point of Care Testing for Urinary Tract Infection in Primary Care (POETIC) study evaluated a modified version of the Flexicult$^{TM}$ SSI-urinary kit a POCT urine culture test that is already in wide use in Denmark. [9,10] Flexicult$^{TM}$ is an overnight point of care test (POCT), which can provide GPs with organism identification, quantification and antibiotic sensitivities within 24 hours. The POETIC trial aimed to determine whether a POCT used at the point of care could improve targeting of antibiotic therapy and patient outcomes in uncomplicated UTI.

Performing urine culture at the point of care reduces delay between obtaining the sample and agar inoculation following receipt of urine in a laboratory, and obtaining a report of the identification, quantification and sensitivity results from the laboratory. However, the degree to which GPs' interpretations of the POCT agree with laboratory interpretation of the POCT results and routine laboratory culture is unknown. We used the opportunity of the POETIC Trial of the effect of the

Flexicult™ POCT in routine care to identify discrepancies between point of care and laboratory urine culture in women presenting to primary care with symptoms of uncomplicated UTI.

## Methods

### *Participants*

All data came from participants of the POETIC randomised controlled trial. [10] Women were recruited from primary care research networks in four countries (Wales, England, Spain and the Netherlands) between June 2013 until September 2014. They were randomised to receive treatment guided by Flexicult™, or standard care. Only data from the participants randomised to the Flexicult™ care arm are included in this analysis. The design and rationale of POETIC trial are described elsewhere.[10] Briefly, eligible patients were adult women aged 18 years and older presenting to primary care with at least one of three key urinary tract symptoms (dysuria, urgency and frequency) and where the GP suspected an uncomplicated UTI. Exclusion criteria at baseline were women who were either terminally ill, were receiving treatment for life-threating cancer, were having severe systemic symptoms or had received antibiotics for UTI within the past four weeks. Informed, written consent was obtained from all the participants. The trial was approved by the Research Ethics Committee for Wales recognised by the United Kingdom Ethics Committee Authority and also approved by the relevant local Governance Committees in the Netherlands and Spain.

### *Primary objective of the present analysis*

Three assessments of urine culture results were available: 1) Primary care clinicians' record of their interpretation of Flexicult™ results; 2) Laboratory staff interpretations of photographs of the Flexicult™ taken by clinicians at the time of their interpretation; and 3) routine microbiology laboratory culture results. The primary objective of the current analysis was to identify potentially clinically important differences in organism identification, quantification, sensitivity and culture growth between the clinicians' interpretation' of Flexicult™, laboratory staff' interpretations of photographs of Flexicult™, and the laboratory culture results.

### *Urine sampling procedures*

Flexicult™ is designed for use in primary care and is essentially an agar plate with higher sides, divided into six sections and containing chromogenic agar. In addition to culturing bacteria (for

bacterial quantification) and identification, antibiotic susceptibility of bacteria is determined through observing growth in sections which are impregnated with commonly used antibiotics (figure 1). The larger section allows for identification and quantitative analysis, and 5 smaller sections for antibiotic susceptibility testing. There were differences in the antibiotics incorporated into the Flexicult™ POCT to reflect local differences in antibiotics commonly prescribed for UTI in each country (figure 1).

Clinicians were provided with face to face training as part of POETIC, a country specific Flexicult™ brochure, and a poster to aid interpretation of results. Mid-stream urine samples were collected using a urine collection device (Peezy Midstream, Forte Medical). A fraction of this urine sample was sent by post in a universal tube containing boric acid to laboratories assigned to the POETIC study in each country, and the remainder was used to inoculate the Flexicult™ POCT culture plate. The results of Flexicult™ were documented by the clinicians after overnight (18-24 hours) incubation in a benchtop incubator at a temperature of 35-37ºC. Bacterial growth was recorded (i.e. no growth, pure growth or mixed growth of an organism, and if mixed growth then presence of predominant growth). Bacterial quantification assessed the number of colonies (less than 15 colonies, 15-20 colonies corresponding to ≤10e3 CFU/mL, ≥20 colonies corresponding to 10e3-10e5 CFU/mL, semi confluent/confluent growth corresponding to ≥10e5 CFU/mL). Clinicians compared the colony colour and morphology of their plate to example illustrations in the Flexicult™ brochure to identify organisms. If bacterial growth was assessed at ≥10e3 CFU/mL of a pure or predominant organism, then clinicians were asked to record antibiotic susceptibility. Clinicians also photographed the Flexicult™ plates at the time of their assessment.

The photographs of Flexicult™ plates were interpreted independently by two experienced UK microbiology laboratory staff blind to clinicians' interpretations and laboratory culture findings. The staff conferred with a senior microbiologist when discrepancies occurred (<1%, usually due to poor resolution in pictures) and provided a consensus result.

Urine samples were processed by culturing 50uL of urine onto chromogenic agar (Oxoid, Poole, UK) using spiral plater (Don Whitley, UK). Plates were incubated for 20 hours then viable counts of all species present taken. Pure or predominant organisms were identified using MALDI-ToF (Bruker,

Germany) with the background organism identification estimated from the chromogenic agar. Antibiotic susceptibility was determined by agar dilution using EUCAST breakpoints.

*Definition of positive UTIs*

There is currently no strict consensus concerning thresholds for a laboratory definition of a UTI. Therefore, several common definitions for a positive microbiological UTI diagnosis were considered for this analysis.

Flexicult™ definition of a UTI:

- ≥10e3 CFU/mL, pure culture of a urinary tract pathogen
- ≥10e3 CFU/mL, predominant growth of urinary tract pathogen in mixture with normal flora

Three Laboratory culture definitions of a UTI:

1. European guidelines definition of a UTI[11]:
   - 10e3 CFU/mL of a uropathogen is diagnostic in women who present with symptoms of acute uncomplicated cystitis
2. Public Health England(PHE)/Health Protection Agency guidelines(HPA) definition[20]
   - ≥10e4 CFU/mL, pure culture of a pathogen *OR*
   - ≥10e5 CFU/mL, mixed growth with one predominant pathogen *OR*
   - ≥10e3 CFU/mL, growth of either *E. coli* or *S. saprophyticus*
3. UK laboratory definition
   - ≥ 10e5 CFU/mL, pure culture of a uropathogen *OR*
   - ≥ 10e5 CFU/mL, predominant culture a uropathogen with 3 log difference between highest and next species

*Statistical analysis*

Urine results were compared in three ways; 1) the routine laboratory culture results versus the clinicians' interpretation of Flexicult™ ('Lab vs Flexicult™'); 2) the clinicians' interpretation of Flexicult™ versus the laboratory staff's interpretation of the Flexicult™ photographs ('Flexicult™ vs Photo'); and, 3) the routine laboratory culture results versus the UK microbiology laboratory staff's interpretation of the Flexicult™ photographs ('Lab vs Photo'). Further exploration focused on the laboratory culture results and the clinicians' interpretation of Flexicult™ results ('Lab vs Flexicult™'). Only cases with complete data in both the groups for that specific variable (i.e. threshold growth, purity of bacterial growth) were used. Flexicult™ defined growth of one organism 10 times greater than any other as predominant, whereas the laboratory used 1000 times greater as predominant. Cross tabulation with Cohen's kappa estimates were compared for the three groups. Concordance of the identified organisms, susceptibility results and agreement in the diagnosis of UTI were only analyzed between the laboratory cultures and the Flexicult™ cultures. Prevalence Adjusted Bias Adjust kappa (PABAK[12]) values were estimated. When only small numbers were available (i.e., n≤10) for a

susceptibility testing results for a particular antibiotic, this antibiotic was excluded for further analysis. To assess significant discrepancies in resistance rates, p-values were calculated using McNemar's test and Bonferroni adjustments were made. Statistical analyses were performed with SPSS version 22.0 (SPSS Inc, Chicago, ILL, USA).

**Results**

In all, 643 patients were recruited into the POETIC Trial, and 325 patients were randomised to the Flexicult$^{TM}$ arm. Results from the Flexicult$^{TM}$ were recorded for 312 (96.0%) participants (Figure 2). All included participants were female, their mean age was 49 years, 85.4% had a history of UTI, 68.6% were from the UK, 27.1% from Spain, and 4.2% from the Netherlands. In the UK and the Netherlands, the Flexicult$^{TM}$ plates were read by either GPs, nurses or other health care professionals. In Spain, the Flexicult$^{TM}$ plates were read only by GPs.

*Purity of growth and bacterial quantification*

The purity of bacterial growth in Flexicult$^{TM}$ was compared with the purity of growth on routine culture media in the laboratory for 294 corresponding urine samples (Table 1). Overall, there was a very low level of inter-rater agreement (Kappa = 0.06 (95%CI 0.000-0.122)). There were particularly marked discrepancies within the mixed growth categories.

There were fewer samples with bacterial counts of ≥10e3 CFU/mL in the laboratory culture (182/276) compared to Flexicult$^{TM}$ (204/276). The concordance between the laboratory culture and Flexicult$^{TM}$ was similar for the urine samples with colony counts of ≤10e3 CFU/mL (34.0%) and colony counts of 10e3-10e5 CFU/mL (34.6%), but concordance was only 58.7% for the urine samples with colony counts of ≥10e5 CFU/mL.

There were important discrepancies in laboratory staff interpretation of the photographs of Flexicult$^{TM}$ plates compared to clinicians' interpretation of Flexicult$^{TM}$ and compared to routine culture results with respect to both purity of growth and bacterial quantification (Supplementary table S1).

The subsequent analyses focuses only on the comparison of the laboratory culture results and the clinicians' interpretation of Flexicult$^{TM}$ results ('Lab vs Flexicult$^{TM}$').

*Organism identification*

In routine laboratory culture, pure or predominant uropathogens were isolated with quantitative counts of ≥10e3 CFU/mL in 124/287 samples. The most commonly isolated species was *E. coli* (75.8%), followed by *S. saprophyticus* (5.6%). In contrast, Flexicult™ identified 200/287 (69.7%) pure or predominant uropathogens with quantitative counts of ≥10e3 CFU/mL. *E. coli* accounted for 58.0% of bacterial species isolated with Flexicult™, followed by *Enterococcus* (20.0%). Overall, 82.9% of pure/predominant *E. coli* with quantitative counts of ≥10e3 isolated by routine culture (reference test) were also identified by Flexicult™.

*Antimicrobial susceptibility results*.

Flexicult™ identified *E. coli* in 63 urine samples (Table 2). Laboratory culture found no resistance to nitrofurantoin, fosfomycin or cefuroxime. Therefore, statistical analysis for laboratory culture and Flexicult™ could not be done. Resistant pathogens were more common according to Flexicult™ compared to laboratory culture. The accuracy of the susceptibility testing in Flexicult™ varied from 81.0 % for amoxicillin/clavulanate to 96.6% for ciprofloxacin using laboratory susceptibility analysis as the reference standard. Kappa scores ranged from 0.49 to 0.60, which indicates a 'moderate' level of agreement. PABAK scores, calculated to adjust for the prevalence and bias, showed 'good' to 'very good' agreement (0.60-0.94) between Flexicult™ and laboratory culture for all the analysed antibiotics.

Amoxicillin and cephalothin susceptibility results were excluded from analysis due to small numbers (n≤10).

*UTI diagnosis*

Flexicult™ resulted in 202 samples (69.9%) being classified as positive for a UTI. The proportion of positive samples on laboratory culture varied according to the definition used, but all definitions resulted in a lower proportion than was identified using Flexicult™. The European guidelines definition[11] identified 190 positive samples (65.7%) as UTI, HPA/PHE definition[13] resulted in 137 (47.4%) positive samples, and the UK laboratory definition resulted in 94 (32.5%) positive samples. Table 3 shows the number of concordant and discordant samples along, with agreement measures.

Flexicult™ identified false positives for UTI of between 21.4-44.3%, and false negatives of between 6.9-17.3%, sensitivity of between 73.7% -78.8% (95%CI 66.8-79.8, 95% CI 71.0-85.3) and specificity of between 34.4-38.1% (95%CI 27.7-41.5, 95%CI 30.4-46.4) when compared to the various laboratory thresholds for UTI. The agreement values were all poor (Table 3).

**Discussion**

The aim of this study was to describe potentially important discrepancies between Flexicult™ POCT urine culture results, their corresponding photograph, and the corresponding laboratory urine culture results for urine samples obtained from women presenting to their primary care practice with symptoms of a UTI. We found that Flexicult™ POCT and laboratory urine routine culture had poor levels of agreement in identifying microbiologically positive urine samples. Flexicult™ tended to overestimate the positivity rate for a urine sample taken for UTI when laboratory culture was used as reference standard. Moreover, we identified important discrepancies regarding bacterial quantification and purity of growth between Flexicult™ and laboratory culture, especially for determining predominant growth. However, Flexicult™ compared to laboratory culture identified the vast majority (82.9%) of E. coli correctly and the susceptibility testing results were reasonably concordant for ciprofloxacin, amoxicillin/clavulanate and trimethoprim. GPs were more likely to overcall the “no growth” result, mainly to denote no significant growth. This did not impact upon diagnosis of UTI.

Blom *et al*.[9] analysed results from Flexicult™ plates compared to laboratory cultures from 121 patients in Danish primary care with suspected UTI, and found an error rate of 16% for quantification and an error rate of <7% for antibiotic susceptibilities. However, purity of growth was not analysed. Bongard *et al*.[14] compared laboratory urine microscopy and culture with findings from UK Flexicult™ plates for 200 urine samples submitted routinely from hospital and primary care patients to a hospital microbiology laboratory. There was an error rate of 16.5% in defining a urine sample UTI positive.[14] This compares to the error of 38.8%-51.2% that we found, which may have arisen because only one observer read all of the plates in the Bongard study compared to many clinicians reading only a small number of plates each in the less controlled environment of clinical practice. Moreover, different

criteria for the laboratory definition of UTI were used. The Danish Flexicult™ study based their definition only on bacterial quantification ≥10e3 CFU/mL, whereas Bongard *et al.* used a bacterial quantification threshold ≥10e5 CFU/mL and also took the purity of the organisms (i.e. pure/predominant) into account. This is in line with our findings that changing the definitions influences the numbers of apparent false negative or false positive results by Flexicult™.

Traditionally, growth of ≥10e5 CFU/mL of uropathogens from urine has been considered to indicate a microbiologically defined UTI. However, previous studies demonstrated that urine samples from symptomatic women with pyuria often contain bacterial growth of ≤10e5 CFU/mL.[15,16]

Three other culture based POC UTI diagnostic tests have been assessed (i.e. Uricult Trio[17], DipStreak[18], Diaslide[19]), which have be shown to have high sensitivity (≥88%) and specific (≥90%) for diagnosing a UTI when compared to laboratory culture, although confidence intervals were not reported and antibiotic susceptibility was not analysed.

Since all patients included in the POETIC trial had symptoms attributable to UTI, the low prevalence of urine samples meeting the HPA/PHE and UK laboratory criteria for UTI is unexpected, and may indicate that laboratory culture results in high false negative rates or that there were other (non-infectious) causes of the symptoms. UTI prevalence similar to Flexicult™ was found when a lower threshold, for example the European guideline definition, was used. Concordance between the laboratory cultures and Flexicult™ for no growth was low (18%), which could be explained by changes in viable bacteria in urine samples between the time the sample was taken and when it was analysed in the laboratory.

Strengths of our study include the sample size, the ability to compare results by various definitions of a UTI on culture, and the independently interpretation of the photographs. There are also several limitations. First, the photographs of Flexicult™ plates were interpreted independently by two experienced UK microbiology laboratory staff blind to clinician's interpretations and laboratory culture findings. However, the GPs were more likely to overcall pure or predominant growth – they were less likely to note the presence of other bacteria in the culture. Microbiologists are trained to look for this and so may have been more adept at identifying mixed cultures. Overcalling pure and predominant

growth would account for the higher UTI prevalence within the GP interpretations. This could lead to over-prescribing of antibiotics in primary care if one assumes that the laboratory results more often both correctly identifies and correctly rules out UTIs. On the other hand, because Flexicult™ is based on fresher urine samples and may be interpreted in conjunction with clinical findings, it may provide a more accurate estimate of true positive and true negative UTIs.[20] Secondly, there were major disagreements between laboratory staff interpretation of Flexicult™ photographs compared to clinicians' interpretation of Flexicult™ and routine culture results. Whilst the reasons for these differences are not clear, they may relate to errors in the clinicians understanding or interpretation as well as the quality of the photographs. Until the reasons for such discrepancies are clearer, photographs of Flexicult™ plates should neither be considered as a reliable diagnostic device nor as a useful tool for quality assurance. Thirdly, predominant growth of uropathogens differed markedly between findings from Flexicult™ and laboratory culture, which may be due the different criteria used for determining predominant growth. Furthermore, Flexicult™ tended to overestimate antibiotic resistance, which could lead to GPs prescribing more broad spectrum antibiotics. However, the differences were not significant and prescribing a broad-spectrum antibiotic instead of a narrow spectrum antibiotic when organisms are resistant could benefit patients in the short term. However, over prescribing of broad spectrum antimicrobials is the most common cause of resistance development. Finally, discrepancies between laboratory culture in five different laboratories may have contributed to the overall lack of concordance. Clinicians who interpreted Flexicult™ plates would have had limited experience in doing so, and clinicians only had approximately an hour of training in Flexicult™ interpretation

## Conclusion and clinical implications

Overall, our findings suggest Flexicult™ used in the context for routine primary care, provides information that differs in important ways from laboratory culture especially regarding quantification. The value of Flexicult™ in diagnosing UTI remains unclear, but we found reasonable evidence for its value in antibiotic susceptibility testing, and this could be used before prescribing antibiotics, for example in deciding on which antibiotic to use for either an immediate or delayed prescription once a decision has been made to consider antibiotics. Future studies that correlate diagnostic test results (at various thresholds) with symptoms and response to treatment are urgently required in order to

determine both the most accurate criteria for defining a positive UTI on urine culture, and to determine whether near patient tests provide a more accurate guide to symptomatic benefit from antibiotic treatment.

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**Supplementary material**
Supplementary Table S1

**Author contribution**
CCB led the funding application, study design and study implementation. SH and CCB led the drafting of this manuscript and all authors contributed to the analysis and interpretation of the findings, and to the drafting of the final paper.

**Declaration**
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Ethical approval: The trial was approved by the Research Ethics Committee for Wales recognised by the United Kingdom Ethics Committee Authority and also approved by the relevant local Governance Committees in the Netherlands and Spain
Conflict of interest: The authors declare that they have no competing interests**.**

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**Table 1 Cross tabulation of GP interpretation of Flexicult™ plate (Flexicult(tm)) versus laboratory culture in respect with the bacterial quantification and purity of growth (either no growth, pure growth or mixed growth)).**
*Abbreviations : pred, predominant*

| **Bacterial growth** *n=294* | **Laboratory** No growth | Mixed *not pred.* | Mixed *pred.* | Pure growth | Total |
|---|---|---|---|---|---|
| **Flexicult™** | | | | | |
| No growth | 8 | 9 | 3 | 15 | 35 |
| Mixed growth *(Not predominant)* | 3 | 4 | 3 | 6 | 16 |
| Mixed growth (*Predominant*) | 10 | 37 | 7 | 37 | 91 |
| Pure growth | 23 | 34 | 11 | 84 | 152 |
| Total | 44 | 84 | 24 | 142 | 294 |
| **Statistical measurements** | | | | | |
| Kappa *(95%CI)* | 0.06 (0.000-0.122) | | | | |
| Error rate | 65% | | | | |

| **Threshold growth** *n=276* | **Laboratory** <10e3 CFU/mL | 10e3-10e5 CFU/mL | >10e5 CFU/mL | Total |
|---|---|---|---|---|
| **Flexicult™** | | | | |
| <10e3 CFU/mL | 32 | 20 | 20 | 72 |
| 10e3-10e5 CFU/mL | 38 | 27 | 23 | 88 |
| ≥10e5 CFU/mL | 24 | 31 | 61 | 116 |
| Total | 94 | 78 | 104 | 276 |
| **Statistical measurements** | | | | |
| Kappa *(95%CI)* | 0.147 (0.061-0.233) | | | |
| Error rate | 57% | | | |

**Table 2 Susceptibility results for both the Flexicult™ test and the laboratory cultures for the cases in which *Escherichia coli* was correctly identified by the Flexicult™.**

| ciprofloxacin n=58 | Laboratory culture Sensitive | Resistant | Total |
|---|---|---|---|
| **Flexicult™** | | | |
| Sensitive | 55 | 0 | 55 |
| Resistant | 2 | 1 | 3 |
| Total | 57 | 1 | 58 |
| Resistance rate Lab: | 2% | | |
| Resistance rate Flex: | 5% *p-value 0.500* | | |
| Sensitivity *(95%CI)* | 100 (2.50-100.0) | | |
| Specificity *(95% CI)* | 96 (87.9-99.6) | | |
| PPV | 33 (0.84-90.6) | | |
| NPV | 100 (93.5-100.0) | | |
| Error rate (%) | 3% | | |
| Kappa *(95%CI)* | 0.49(-0.113-1.087) | | |
| PABAK | 0.94 | | |

| amoxi/clavula n=58 | Laboratory culture Sensitive | Resistant | Total |
|---|---|---|---|
| **Flexicult™** | | | |
| Sensitive | 39 | 2 | 41 |
| Resistant | 9 | 8 | 17 |
| Total | 48 | 10 | 58 |
| Resistance rate Lab: | 17% | | |
| Resistance rate Flex: | 29% *p-value 0.065* | | |
| Sensitivity *(95%CI)* | 80 (44.4-97.5) | | |
| Specificity *(95% CI)* | 81 (67.4-91.1) | | |
| PPV | 47 (23.0-72.2) | | |
| NPV | 95 (83.5-99.4) | | |
| Error rate (%) | 19% | | |
| Kappa *(95%CI)* | 0.48(0.227-0.732) | | |
| PABAK | 0.62 | | |

| nitrofurantoin n=52 | Laboratory culture Sensitive | Resistant | total |
|---|---|---|---|
| **Flexicult™** | | | |
| Sensitive | 47 | 0 | 47 |
| Resistant | 5 | 0 | 5 |
| Total | 52 | 0 | 52 |
| Resistance rate Lab: 0% | | | |
| Resistance rate Flex: 10% | | | |
| Statistical measurements: - * | | | |

| trimethoprim n=35 | Laboratory culture Sensitive | Resistant | total |
|---|---|---|---|
| **Flexicult™** | | | |
| Sensitive | 27 | 3 | 30 |
| Resistant | 1 | 4 | 5 |
| Total | 28 | 7 | 35 |
| Resistance rate Lab: 20% | | | |
| Resistance rate Flex: 14 % *p-value 0.625* | | | |
| Sensitivity *(95%CI)* | 57.1 (18.4-90.1) | | |
| Specificity *(95% CI)* | 96.4 (81.6-99.0) | | |
| PPV | 80.0 (28.4-99.5) | | |
| NPV | 90.0 (73.5-97.9) | | |
| Error rate (%) | 11% | | |
| Kappa *(95%CI)* | 0.60 (0.249-0.951) | | |
| PABAK | 0.78 | | |

| fosfomycin n=21 | Laboratory culture Sensitive | Resistant | Total |
|---|---|---|---|
| **Flexicult™** | | | |
| Sensitive | 18 | 0 | 18 |
| Resistant | 3 | 0 | 3 |
| Total | 21 | 0 | 21 |
| Resistance rate Lab: 0% | | | |
| Resistance rate Flex: 14 % | | | |
| Statistical measurements: - * | | | |

| cefuroxime n=20 | Laboratory culture Sensitive | Resistant | Total |
|---|---|---|---|
| **Flexicult™** | | | |
| Sensitive | 17 | 0 | 17 |
| Resistant | 3 | 0 | 3 |
| Total | 20 | 0 | 20 |
| Resistance rate Lab: 0% | | | |
| Resistance rate Flex: 15% | | | |
| Statistical measurements: - * | | | |

*Abbreviations: Lab, Laboratory; Flex, Flexicult™; PPV, positive predictive value; NPV, negative predictive value, PABAK, Prevalence Adjusted Bias Adjust Kappa. * Due to no resistance rate in laboratory culture further statistics are not calculated.*

**Table 3 Cross tabulation of Flexicult™ versus laboratory cultures (with the different thresholds) in determining urinary tract infections (n=289).**
*3A Flexicult™ versus EUCAST >10e3 definition of UTI; 3B Flexicult™ versus PHE/HPA definition of UTI; 3C Flexicult™ versus UK laboratory definition of UTI.*

| **UTI Yes/No n=289** | **European >10e3 definition** | | |
|---|---|---|---|
| | Yes UTI | No UTI | Total |
| **Flexicult™** | | | |
| Yes UTI | 140 48%) | **62 (21%)** | 202 |
| No UTI | **50 (17%)** | 37 (13%) | 87 |
| Total | 190 | 99 | 289 |
| **Statistical measurements** | | | |
| Sensitivity *(95%CI)* | 73.7 *(66.8-79.8)* | | |
| Specificity *(95% CI)* | 37.4 *(27.9-47.7)* | | |
| PPV | 69.3 (62.5-75.6) | | |
| NPV | 42.5 (32.0-53.6) | | |
| Error rate (%) | 39% | | |
| Kappa *(95%CI)* | 0.11(-0.008-0.232) | | |
| PABAK | 0.22 | | |

**3A**

| **UTI Yes/No n=289** | **PHE/HPA definition** | | |
|---|---|---|---|
| | Yes UTI | No UTI | Total |
| **Flexicult™** | | | |
| Yes UTI | 108 (37%) | **94 (33%)** | 202 |
| No UTI | **29 (10%)** | 58 (20%) | 87 |
| Total | 137 | 152 | 289 |
| **Statistical measurements** | | | |
| Sensitivity *(95%CI)* | 78.8 *(71.0-85.3)* | | |
| Specificity *(95% CI)* | 38.1 *(30.4-46.4)* | | |
| PPV | 53.5 (46.3-60.5) | | |
| NPV | 66.7 (55.8-76.4) | | |
| Error rate (%) | 43% | | |
| Kappa *(95%CI)* | 0.17 *(0.07-0.27)* | | |
| PABAK | 0.14 | | |

**3B**

| **UTI Yes/No n=289** | **UK laboratory definition** | | |
|---|---|---|---|
| | Yes UTI | No UTI | Total |
| **Flexicult™** | | | |
| Yes UTI | 74 (26%) | **128 (44%)** | 202 |
| No UTI | **20 (7%)** | 67 (23%) | 87 |
| Total | 94 | 195 | 289 |
| **Statistical measurements** | | | |
| Sensitivity *(95%CI)* | 78.7 *(69.1-86.5)* | | |
| Specificity *(95% CI)* | 34.4 *(27.7-41.5)* | | |
| PPV | 36.6 (30.0-43.7) | | |
| NPV | 77.0 (66.8-85.4) | | |
| Error rate (%) | 51% | | |
| Kappa *(95%CI)* | 0.10 *(0.02-0.18)* | | |
| PABAK | 0.02 | | |

**3C**

**Abbreviations:** *PHE/HPA, Public Health England/Health Protection Agency;* UTI, urinary tract infection; PPV, positive predictive value; NPV, negative predictive value, PABAK, Prevalence Adjusted Bias Adjust Kappa.

**Figure 1. The UK Flexicult SSI-Urinary Kit**
** Spain: Fosfomycin instead of Trimethoprim, Cefuroxime instead of Cephalothin*
*** Netherlands: Amoxicillin instead of Cephalothin*

**Figure 2. Flowchart of the cases evaluated in the study**
*Abbreviations: CRF, case report form; UTI, urinary tract infection.*